Analysis of the Three Most Common MEFV Mutations in 412 Patients with Familial Mediterranean Fever

Nurit Zaks MD1, Yael Shinar Phd1, Shai Padeh MD1, Merav Lidar MD1, Adam Mor MD1, Irena Tokov MD1, Mordechai Pras MD1, Pnina Langevitz MD1, Elon Pras MD2 and Avi Livneh MD1

1Heller Institute of Medical Research and 2Danek Gartner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer, Israel Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Key words: familial Mediterranean fever, MEFV gene, mutations, amyloidosis

Abstract

Background: Familial Mediterranean fever is an autosomal recessive disease characterized by recurrent attacks of fever and serositis. The disease is caused by mutations in the MEFV gene, presumed to act as a down-regulator of inflammation within the polymorphonuclear cells.

Objectives: To present the results of 412 FMF patients genotyped for three MEFV mutations, M694V, V726A and E148Q.

Results: The most frequent mutation, M694V, was detected in 47% of the carrier chromosomes. This mutation, especially common among North African Jewish FMF patients, was not found in any of the Ashkenazi (Eastern European origin) patients. Overall, one of the three mutations was detected in 70% of the carrier chromosomes. M694V/M694V was the most common genotype (27%), followed by M694V/V726A (16%). The full genotype could be assessed in 57% of the patients, and one mutation causing mutation in an additional 26%. Only one patient with the E148Q/E148Q genotype was detected despite a high carrier rate for this mutation in the Jewish population, a finding consistent with a low penetrance of this genotype. The M694V/M694V genotype was observed in 15 patients with amyloidosis compared to 4 amyloidosis patients with other genotypes (P < 0.0001).

Conclusions: Because of low penetrance and as yet other undetermined reasons, mutation analysis of the most common MEFV mutations supports a clinical diagnosis in only about 60% of patients with definite FMF.

IMA 2003:5:585–588

For Editorial see page 592

Familial Mediterranean fever is an autosomal recessive disease characterized by recurrent episodes of fever accompanied by sterile peritonitis, pleuritis, arthritis, and a typical rash termed erythema [1]. Renal amyloidosis type AA is the most devastating manifestation of the disease, and in the past was a major cause of morbidity and mortality among FMF patients [1]. In some patients, amyloidosis is the only manifestation of the disease (phenotype II). Prophylactic daily colchicine, a treatment modality introduced in the mid 1970s, has altered the natural history of this disease, preventing the occurrence of attacks and the development of amyloidosis [2,3]. FMF is highly prevalent among Middle Eastern populations, especially in North African Jews and Iraqi Jews [4], Armenians [5], Middle Eastern Arabs [6] and Anatolian Turks [7]. The disease is also found in Ashkenazi Jews (Eastern European origin), although it is less common [8].

In 1997, using a positional cloning approach, two groups cloned the FMF gene (MEFV). The gene, which lies in chromosome 16p [9], is composed of 10 exons and encodes a 781 amino acid protein. This protein – named pyrin by the International FMF Consortium [10] and marionostin by the French FMF Consortium [11] – is expressed in polymorphonuclear cells and in activated macrophages. Pyrin’s mode of action is still unclear, but it is presumed to act as a down-regulator of inflammation. Thirty disease-associated mutations have been identified so far in MEFV [12].

Screening of a control population for MEFV mutations revealed an extremely high gene frequency among North African Jews, Iraqi and Ashkenazi Jews, with carrier rates of 20–40% [13-15]. This high carrier rate has highlighted the possibility of a selective advantage for FMF heterozygotes.

In the last 3 years we have routinely screened patients diagnosed with FMF for three of the most common mutations, M694V, V726A and E148Q. In this report we present the results of mutation analysis in 412 patients clinically diagnosed as suffering from definite FMF.

Materials and Methods

Patients and DNA samples
Since 1998, we offer MEFV genetic analysis to all patients arriving at our FMF clinic for diagnosis of FMF. This report includes the first 412 patients with definite FMF diagnosed according to established criteria [16], who undertook this test. Three milliliters of blood were drawn from each patient and DNA was extracted according to standard procedures.

Mutation analysis
The E148Q, M694V and V726A mutations create restriction sites for the enzymes BstNI, HphI and AluI, respectively. Three segments containing these sites were amplified using standard polymerase...
chain reaction procedure and the forward and reverse primers: 5'-GGCTGAAGACTCCACACCC-3' and 5'-AGGCCCTCCAGGCGCTCTC-3', 5'-ACTCTGTCGCGAATGGCTACTGGGAGATGAAATG-3' and 5'-CTCGAGCCCCCATGGGGAACTGGAGC-3', 5'-ACCAGCTGTCATTAAAGGAGCGCCCAGAGG-3' and 5'-GAAGATAGGGTGAAGGGTCCAGAAGAGCAGCTGAC-3', respectively.

PCR products were digested with the appropriate enzymes from the above, electrophoresed on a gradient (4–20%) polyacrylamide gel and stained with ethidium bromide.

**Statistical analysis**
Results were analyzed with the chi-square test.

**Results**
The ethnic composition of the patients analyzed is shown in Figure 1. The largest ethnic group was North African Jews, followed by Iraqi Jews. Most of the patients who appear under 'Others' are of mixed origin; a minority comprises Arab and Druze. The results of the mutation analysis in the 824 carrier chromosomes are shown in Table 1. M694V was by far the most common mutation found in this cohort of patients, followed by V726A. Two mutations on the same carrier chromosome (complex allele) were found in seven carrier chromosomes (1%) of Ashkenazi and Druze patients. The distribution of genotypes in the 412 patients is shown in Table 2. The most common genotype was the M694V/M694V (27%), followed by M694V/V726A (16%). Despite the higher carrier rate of the E148Q mutation in the control Jewish population, only one patient with the E148Q/E148Q genotype was detected. The full genotype could be assessed in 57% of the patients. In an additional 26% of the patients, one disease-causing mutation could be determined. Only in 16% of the patients were none of the three mutations identified. Table 3 stratifies the patients in whom the complete genotype could be determined according to their ethnic origin. The largest ethnic group screened for MEFV mutations was the North African Jewish patients (n=140). Among them, the M694V/M694V genotype was found in 50% of the patients and M694V/E148Q in an additional 10%. Overall, the complete genotype could be assessed in 63% of the North African Jewish patients. The V726A mutation was relatively rare among patients of this ethnic group. Among Iraqi Jews, the second largest ethnic group consisting of 43 patients, the M694V/V726A genotype was the most common (28%), followed by M694V/M694V (9%). The V726A/V726A was the most common genotype found in the 21 Ashkenazi Jewish patients (29%), followed by the E148Q-V726A/V726A genotype (14%). Interestingly, no M694V alleles were detected in this population group.

Of the 412 patients analyzed in this study 19 were diagnosed with amyloidosis. Fifteen amyloidosis patients were detected among 110 patients with the M694V/M694V genotype, compared to only 4 patients with other genotypes (P < 0.0001). Genotypes in these patients included M694V/V726A, M694V/Unknown, V726A-E148Q/V726A, and V726A-E148Q/V726A-E148Q.

**Table 1. Distribution of MEFV mutations in 412 FMF patients**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>No. of chromosomes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V</td>
<td>391</td>
<td>47%</td>
</tr>
<tr>
<td>V726A</td>
<td>122</td>
<td>15%</td>
</tr>
<tr>
<td>E148Q</td>
<td>58</td>
<td>7%</td>
</tr>
<tr>
<td>V726A-E148Q</td>
<td>7</td>
<td>1%</td>
</tr>
<tr>
<td>Total</td>
<td>824</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 2. Genotype distribution in 412 FMF patients**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of patients</th>
<th>%</th>
<th>No. of mutations</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V/M694V</td>
<td>110</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V726A/V726A</td>
<td>14</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E148Q/E148Q</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M694V/V726A</td>
<td>64</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M694V/E148Q</td>
<td>29</td>
<td>7</td>
<td>2</td>
<td>234</td>
</tr>
<tr>
<td>V726A/E148Q</td>
<td>10</td>
<td>2</td>
<td>4</td>
<td>110</td>
</tr>
<tr>
<td>V726A-E148Q/V726A</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>412</td>
<td>100</td>
<td>2 or 0</td>
<td>412</td>
</tr>
</tbody>
</table>

? = undetermined

**Table 3. Fully characterized genotypes stratified according to ethnic distribution**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>North African Jews (n=140)</th>
<th>Iraqi Jews (n=43)</th>
<th>Ashkenazi Jews (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>M694V/M694V</td>
<td>69</td>
<td>50%</td>
<td>4</td>
</tr>
<tr>
<td>V726A/V726A</td>
<td>0</td>
<td>0%</td>
<td>2</td>
</tr>
<tr>
<td>E148Q/E148Q</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>M694V/V726A</td>
<td>4</td>
<td>3%</td>
<td>12</td>
</tr>
<tr>
<td>M694V/E148Q</td>
<td>14</td>
<td>10%</td>
<td>1</td>
</tr>
<tr>
<td>V726A/E148Q</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>V726A-E148Q/V726A</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>63%</td>
<td>20</td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction
Discussion
In this report we present the largest series of mutation analysis in
FMF patients. The patients were screened for three mutations,
which account for 70% of the carrier chromosomes. M694V was the
most common mutation found in this series, accounting for 47% of
the carrier chromosomes. M694V/M694V, the most prevalent
genotype, was especially common among the North African Jewish
patients. This genotype has been previously linked to severe
disease [17–21] and therefore it is not surprising to find it in the
North African Jewish FMF population, which suffers from a severe
disease. Conversely, the M694V mutation is very rare in Ashkenazi
FMF patients [21,22], contributing to a milder phenotype in this
population.

The ethnic distribution of the patients is highly reflective of the
prevalence of FMF in the Israeli population, common in North
African and Iraqi Jews, and rare in Ashkenazi Jews. This distribution
is in striking contrast to the distribution of MEVF mutations found
in normal controls. Recent studies have shown MEVF carrier rates
of 20−40% in North African, Iraqi and Ashkenazi Jews [13–15,22]. The
low prevalence of FMF in Ashkenazi Jews can be explained by the
low penetrance of most of the mutations common in this ethnic
group. For example, the carrier rate for E148Q in Ashkenazi controls
was 7–12% [13–15,22], yet in this study no Ashkenazi patients with
the E148Q/E148Q genotype were detected, a finding consistent with
the low penetrance of this mutation, or with the suggestion that
E148Q is a single nucleotide polymorphism and not a disease-
causing mutation [23]. Of note, however, is the finding that patients
with the E148Q/E148Q genotype do exist and actually express a
severe phenotype [24], and that a combination of E148Q with other
mutations can cause symptomatic disease, as indicated by the 29
patients bearing the M694VE148Q genotype. Sometimes E148Q
can appear on the same chromosome with another mutation,
V276A. This rare allele, associated with a more severe phenotype
and a relatively high penetrance [21,22,25], was found in Ashkenazi
and in Druze patients. Previously, we found another rare mutation
common to Ashkenazi Jews and Druze in SLC3A1, a gene associated
with cystinuria [26]. These common mutations raise the possibility
of a common ancestor to the two populations.

Fifteen of the 19 patients with amyloidosis were M694V
homozygous, confirming the association previously shown between
this genotype and amyloidosis [18–20]. The finding of four other
patients with amyloidosis emphasizes the fact that this dreadful
complication can potentially develop with almost any genotype.
The only known factor that can prevent amyloidosis is daily
prophylactic colchicine, and patients with the M694VE694V
genotype are therefore strongly encouraged to adhere to the
treatment protocol.

The finding in our study of FMF patients who carry only one or
none of the common mutations is probably mainly related to the
limited genetic evaluation conducted, which includes only 3 of
the 30 mutations already identified. Yet, a thorough genotyping,
using sequence analysis, of the whole MEVF still leaves us with a large
population (15–20%) of FMF patients with unmutated MEVF or with
only one mutated allele. This suggests that other genes may be
involved in FMF expression.

A third population, not included in the study but which does
merit mentioning, consists of patients who carry two FMF alleles
yet do not express FMF manifestations (phenotype III) [15]. Based
on the 20–40% carrier rate and direct screening of the Israeli
population for double MEVF mutations, this forms the largest FMF
group in Israel, estimated at around 50,000 individuals. For
comparison, the number of patients with overt FMF is around
10,000. Since the results of the present study reflect only
symptomatic patients, the subsequent diagnostic, clinical,
therapeutic and epidemiologic conclusions may be misleading.

The exact role of molecular analysis in the diagnosis of FMF
remains to be established, even in the asymptomatic patient group
[27]. Genetic testing will probably turn out to be most effective in
patients with an uncertain diagnosis, where two mutations in MEVF
would highly support the diagnosis of FMF [27]. In patients with
typical symptoms and a good response to colchicine, genetic
testing may confirm the diagnosis, but a negative result will not
change the clinical decision and management [27].

References
3. Zemer D, Pras M, Sohar E, Modan M, Cabil S, Gafni I. Colchicine in
the prevention and treatment of the amyloidosis of familial Mediterranean
4. Pras M, Bronshøj N, Zemer D, Gafni I. Variable incidence of
amyloidosis in familial Mediterranean fever among different ethnic
6. Barakat MH, Karnick AM, Majeed HWA, El-Sobki NI, Fenech FF. Familial
Mediterranean fever (recurrent hereditary polyserositis) in Arabia: a study
7. Ozlemir AL, Solmen C. Familial Mediterranean fever among the Turkish
inheritance in two families with familial Mediterranean fever. Am J Med
familial Mediterranean fever to the short arm of chromosome 16. N Engl J
10. The International FMF Consortium. Ancient missense mutations in a
new member of the RoRet gene family are likely to cause familial
12. Touitou I. The spectrum of familial Mediterranean fever mutations. Eur J
for familial Mediterranean fever in various Jewish ethnic groups. Eur J
Mediterranean fever: prevalence, penetrance and genetic drift. Eur J
Jewish ethnic groups in Israel: high frequency of carrier and phenotype
III states and absence of a perceptible biological advantage for the
Capsule

Serotonin transporter and depression

Stressful life events such as the loss of a job can lead to depression, but not everyone shows this response. A study of a large group of young adults in New Zealand by Caspi et al. provides evidence that stress is more likely to cause depression in individuals who carry a particular allelic variant of the gene encoding the serotonin transporter, a protein that controls serotonin levels at brain synapses. These results reinforce the emerging view that mental illness and other complex diseases cannot always be explained by genetic or environmental factors alone, but more likely arise from an interaction between the two.

Science 2003;301:386
E. Israeli

Capsule

Avoiding protein pile-ups

The glomerular basement membrane (GBM) is a filtration barrier in the kidney that permits the loss of excess water while preventing the loss of valuable proteins. Several human renal diseases are characterized by an accumulation of immune complexes in the GBM, but Kim et al. suggest that this condition may not be caused by immune dysfunction associated with disease pathology. Rather, the authors observed abnormal protein accumulation in the GBM of mice deficient in CD2AP, an adaptor protein expressed in podocytes that comprise the GBM. CD2AP-deficient podocytes were defective in degrading endocytosed material. Susceptibility to human renal disease may be determined in part by the intrinsic capacity of the GBM to clear proteins that the kidney normally encounters.

Science 2003;300:1298